

From the Department of Histology, University of Lund,  
Lund, Sweden

STUDIES ON ANATOMICAL,  
FUNCTIONAL AND PLASTIC PROPERTIES  
OF CENTRAL SEROTONINERGIC NEURONS.

By

LEIF WIKLUND

Lund 1980



From the Department of Histology, University of Lund, Lund, Sweden

STUDIES ON ANATOMICAL, FUNCTIONAL AND PLASTIC  
PROPERTIES OF CENTRAL SEROTONINERGIC SYSTEMS

by

Leif Wiklund





The present inquiry is based on the following sources:

- I. Wilentz, L. Development of secondary somatosensory cells and the presynaptic innervation of the secondary motor cortex in the rat. *Cell Tiss. Res.* 125 (1974) 231-261.
- II. Wilentz, L., A. Skolnik and S. Selyanko. The histological localization of the inhibitory effect 1. Convergence with the direct spinal efferents in the area projecting to the cerebellar vermal lobes. *Brain Res.* 131 (1977) 1-27.
- III. Selyanko, S., A. Skolnik and L. Wilentz. The histohistochemical localization of the inhibitory effect 2. Referred to histohistochemical changes. *Brain Res.* 131 (1977) 23-37.
- IV. Wilentz, L., S. J. Leinberg, L. Wilentz, L. Leinberg and M. G. Selyanko. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 38-55.
- V. Wilentz, L. and S. Selyanko. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 56-67.
- VI. Wilentz, L. and S. Selyanko. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 68-79.
- VII. Selyanko, S., L. Wilentz, L. Leinberg and M. G. Selyanko. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 80-91.
- VIII. Selyanko, S., L. Wilentz, L. Leinberg and M. G. Selyanko. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 92-103.
- IX. Wilentz, L. and A. Skolnik. Histohistochemical changes in the area projecting to the cerebellar vermal lobes and the area projecting to the rat's cerebellar lobes. *Brain Res.* 131 (1977) 104-115.

To my parents





The present summary is based on the following papers:

- I. Wiklund, L. Development of serotonin-containing cells and the sympathetic innervation of the habenular region in the rat brain. *Cell Tiss. Res.* 155 (1974) 231-243.
- II. Wiklund, L., A. Björklund and B. Sjölund. The indolaminergic innervation of the inferior olive. 1. Convergence with the direct spinal afferents in the areas projecting to the cerebellar anterior lobe. *Brain Res.* 131 (1977) 1-21.
- III. Sjölund, B., A. Björklund and L. Wiklund. The indolaminergic innervation of the inferior olive. 2. Relation to harmaline induced tremor. *Brain Res.* 131 (1977) 23-37.
- IV. Møllgård, K., J.J. Lundberg, L. Wiklund, L. Lachenmayer and H.G. Baumgarten. Morphological consequences of serotonin neurotoxin administration: Neuron-target cell interaction in the rat subcommissural organ. *Ann.N.Y. Acad. Sci.* 305 (1978) 262-288.
- V. Møllgård, K. and L. Wiklund. Serotonergic synapses on ependymal and hypendymal cells of the rat subcommissural organ. *J. Neurocytol.* 8 (1979) 445-467.
- VI. Wiklund, L. and K. Møllgård. Neurotoxic destruction of the serotonergic innervation of the rat subcommissural organ is followed by reinnervation through collateral sprouting of non-monoaminergic neurons. *J. Neurocytol.* 8 (1979) 469-480.
- VII. Léger, L., L. Wiklund, L. Descarries and M. Persson. Description of an indolaminergic cell component in the cat locus coeruleus: A fluorescence histochemical and radioautographic study. *Brain Res.* 168 (1979) 43-56.
- VIII. Björklund, A. and L. Wiklund. Mechanisms of regrowth of the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy. I. Biochemical correlates. *Brain Res.* (1980) in press.
- IX. Wiklund, L. and A. Björklund. Mechanisms of regrowth in the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy. II. Fluorescence histochemical observations. *Brain Res.* (1980) in press.

The present summary is based on the following papers:

- I. Willund, L. Development of serotonin-containing cells and the sympathetic innervation of the abdominal region in the rat brain. *Cell Tiss. Res.* 155 (1974) 231-243.
- II. Willund, L., A. Björklund and B. Ståhl. The indoleaminergic innervation of the inferior olive. I. Convergence with the direct spinal efferents in the olive projecting to the cerebellar anterior lobe. *Brain Res.* 131 (1977) 1-21.
- III. Ståhl, B., A. Björklund and L. Willund. The indoleaminergic innervation of the inferior olive. 2. Relation to formative reduced trigone. *Brain Res.* 131 (1977) 23-37.
- IV. Møllgård, K., J. L. Lundberg, L. Willund, L. Löcherer and H. G. Baumgarten. Morphological consequences of serotonergic neurotoxin administration: Neurotoxic cell interaction in the rat midbrain. *Ann. N.Y. Acad. Sci.* 308 (1978) 247-260.
- V. Møllgård, K. and L. Willund. Serotonergic synapses on identified and unidentified cells in the rat midbrain. *J. Neurocytol.* 8 (1979) 445-465.
- VI. Willund, L. and L. Møllgård. Presynaptic destruction of the indoleaminergic innervation of the rat midbrain: effects of reserpine and restoration through collateral sprouting of serotonergic neurones. *J. Neurocytol.* 8 (1979) 467-480.
- VII. Ståhl, B., L. Willund, L. Møllgård and B. Larsson. Distribution of an indoleaminergic cell component in the rat brain. *Brain Res.* 155 (1977) 43-54.
- VIII. Björklund, A. and L. Willund. Mechanisms of reserpine-induced changes in serotonergic system following 3,4-dihydroxyphenylamine-induced damage. *J. Neurocytol.* 8 (1979) 1-10.
- IX. Willund, L. and A. Björklund. Morphological changes in the indoleaminergic system following 3,4-dihydroxyphenylamine-induced damage. II. Fluorescence histochemical observations. *Brain Res.* 155 (1977) 55-64.



- X. Wiklund, L., L. Léger and M. Persson. Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *J. Comp. Neurol.* (submitted for publication).
- XI. Wiklund, L., L. Descarries and K. Møllgård. Regeneration of serotonergic innervation after 5,6-dihydroxytryptamine studied with high resolution radioautography in the rat dorsal accessory olive. *J. Neurocytol.* (submitted for publication).



## CONTENTS

|                                                                             | Page |
|-----------------------------------------------------------------------------|------|
| GENERAL INTRODUCTION                                                        | 6    |
| MATERIALS AND METHODS                                                       | 9    |
| RESULTS AND COMMENTS                                                        | 14   |
| A. Distribution of serotonergic cell bodies.                                | 14   |
| B. Serotonergic innervation of the inferior olive.                          | 17   |
| 1. Serotonergic innervation.                                                | 17   |
| 2. Harmaline induced tremor.                                                | 18   |
| 3. Serotonin syndrome.                                                      | 19   |
| 4. Neuromodulatory role of serotonin in inferior olive.                     | 20   |
| C. Serotonergic innervation of the rat subcommissural organ.                | 23   |
| D. Plasticity of serotonergic connections after neurotoxic lesions.         | 25   |
| 1. Mode of termination and course of neuroplasticity.                       | 25   |
| 2. Observations on the regeneration of the bulbospinal serotonergic system. | 27   |
| PERSPECTIVES                                                                | 29   |
| ACKNOWLEDGEMENTS                                                            | 30   |
| REFERENCES                                                                  | 32   |





## GENERAL INTRODUCTION

Serotonin was originally detected in central nervous tissue by Twarog and Page (1953). Differential distribution of serotonin within CNS was first reported by Amin et al. (1954), and Heller et al. (1962) managed to demonstrate decreases in brain serotonin content after lesions of the medial forebrain bundle. The early attempts to determine the anatomy of postulated serotonin neurons was, however, hampered by the inability to relate the biochemical findings with any known neuroanatomical system. Therefore, the development of sensitive fluorescence histochemical techniques visualizing minute quantities of monoamines, including serotonin, (Falck, 1962; Falck et al., 1962) provided a major breakthrough for all further investigations of central serotonergic systems. Using the Falck-Hillarp fluorescence histochemical technique, Dahlström and Fuxe (1964) demonstrated serotonergic cell bodies concentrated in the raphe nuclei of the rat brain stem, and defined nine different groups of serotonergic cells (B 1 to B 9). Serotonergic nerve terminals were demonstrated in wide-spread areas of CNS (Fuxe, 1965). Studies combining intracerebral lesions with fluorescence histochemistry or biochemical determinations of serotonin content, uptake or biosynthetic enzymes have confirmed that the serotonergic cell bodies in the brain stem are the origin of the serotonergic terminals in forebrain as well as spinal cord (e.g. Dahlström and Fuxe, 1965; Andén et al., 1966; Ungerstedt, 1971; Björklund et al., 1973a; Lorens and Guldberg, 1974; Kellar et al., 1977; Palkovits et al., 1977; Van de Kar and Lorens, 1978). Recently, studies employing anterograde radioautographic or retrograde horseradish peroxidase tracing technique have given further insight into the complex pattern of serotonergic connections (e.g. Segal and Landis, 1974 a, b; Kuypers and Maisky, 1975; Bobillier et al., 1976, 1979; Taber et al., 1977; Azmitia and Segal, 1978; Basbaum et al., 1978, 1979; Jacobs et al., 1978; Moore et al., 1978; Tohyama et al., 1979). Thus, it appears that the rostral B 7-9 groups of serotonergic cells project to forebrain areas, the most caudal B 1-3 groups are origin of spinal serotonergic innervation, while all serotonergic groups may contribute to the innervation of different parts of brain stem and cerebellum. A few studies have concerned themselves with the input to raphe nuclei (e.g. Moolenaar et al., 1976; Wang et al., 1976; Aghajanian and Wang, 1977; Sakai et al., 1977; Gallager and Pert, 1978).





But, generally, much less is known about the afferent connections of serotonergic neurons than their efferent projections.

The ultrastructure of serotonergic innervation has recently become subject to intensive investigation (e.g. Chan-Palay, 1975; Descarries et al., 1975; Calas et al., 1976; Bouchaud and Arluison, 1977; Léger and Descarries, 1978; Arluison and De La Manche, 1980; and our papers IV, V, and XI). Serotonergic terminals in different regions demonstrate surprising variation of organelle content: some contain small clear vesicles, others microcanalicular and/or microvesicular organelles, and some are dominated by large granular vesicles. Another important feature is that in many regions the serotonergic fibers terminate with formation of very few classic synaptic junctions (marked by ultrastructurally demonstrable pre- and postmembraneous specializations). It has been suggested that this "non-synaptic" mode of termination may be related to particular dynamic functional properties involving continuous reshaping of the terminal plexa (Descarries et al., 1975; Beaudet and Descarries, 1978). We have attempted to correlate these particular termination properties with the regenerative capacities demonstrated after selective neurotoxic lesions of serotonergic connections (papers VI and XI).

Serotonergic neurons are considered to play an important role in many CNS functions, such as sleep-wakefulness (for review, see Jouvet, 1972), thermoregulation (Feldberg and Myers, 1964; Komiskey and Rudy, 1977), modulation of pain sensitivity (for review and references, see Messing and Lytle, 1977; Griersmith and Duggan, 1980) neuroendocrine control (for recent reviews, see Baumgarten et al., 1978; Krulich, 1979) and spinal reflexes (e.g. Andén et al., 1964; Barasi and Roberts, 1974). A number of investigators have studied the effects of serotonin on different target cells. The experiments have usually involved iontophoretic application of serotonin onto the target cell with multibarrel electrode technique, but some investigators have also stimulated the raphe nuclei of origin. Virtually all investigators have assessed the effects of serotonin (or serotonergic innervation) by extracellular recording and computerized spike frequency analysis. Most commonly, serotonin has been found to result in decreased firing of target cells (e.g. Segal, 1975; Sastry and Phillips, 1977b; Wang and Aghajanian, 1977; Reader et al., 1979), but increased spontaneous firing (Boakes et al., 1970) and facilitation of induced activity (McCall and Aghajanian, 1979) have been reported in certain regions. An interesting observation is that the state of activity may influence the type of action



serotonin has on its target cells (Szabadi et al., 1977). Due to the techniques generally used only little is known about the ionic and/or biochemical mechanisms involved in the serotonin mediated effects. The inhibitory effect of serotonin on spinal motoneurons has by Engberg et al. (1974) been shown to involve hyperpolarization and increased membrane resistance. The inhibitory effect on cortical neurons has been suggested to involve activation of a  $\text{Na}^+$ ,  $\text{K}^+$  -ATPase dependent electrogenic pump (Sastry and Phillis, 1977a), and it has been speculated that  $\text{Ca}^{++}$  may serve a "messenger" function mediating the serotonin receptor response (Phillis et al., 1973; for discussion see Reader et al., 1979). Serotonin sensitive adenylate cyclase has been reported in the rat brain (von Hungen et al., 1975), but no definite evidences indicate that cyclic nucleotides mediate the physiologically recorded serotonin receptor effects. At last it must be pointed out that it is often difficult to integrate the single unit findings with the holistic theories of serotonergic function..





## MATERIAL AND METHODS

### ANIMALS

Female and male adult, juvenile and neonatal (own breeding) rats of the Sprague-Dawley strain, and young cats of the European short haired race have been used in the course of these studies.

### ADMINISTRATION OF NEUROTOXIC DRUGS

Rats were injected stereotaxically into the lateral ventricle with either of the following: 50 or 75  $\mu$ g 5,6-dihydroxytryptamine (5,6-DHT, Regis), 150  $\mu$ g 5,7-dihydroxytryptamine (5,7-DHT), or 250  $\mu$ g 6-hydroxydopamine (Hässle). In each case the neurotoxic drug was dissolved in 20  $\mu$ l sterile saline containing 0.2 mg ascorbic acid per ml, as antioxidant. In some experiments, the rats to receive 5,7-DHT were pretreated with desipramine 25 mg/kg, i.p., 30-60 minutes before, in order to protect noradrenergic neurons. Normal untreated rats and rats injected in the ventricle with 20  $\mu$ l saline served as controls. (For detailed account of the properties of these neurotoxic drugs, see Baumgarten et al., 1977).

### FLUORESCENCE HISTOCHEMISTRY

Animals used for fluorescence histochemical detection of monoamines were usually pharmacologically pretreated to increase intraneuronal serotonin content. Rats were administered either of the following: a) nialamide 300 mg/kg, i.p. b) nialamide 300 mg/kg one hour before L-tryptophan 100 mg/kg c) chloral hydrate 200-400 mg/kg 10 minutes before nialamide 300 mg/kg, and after another 15 minutes L-tryptophan 100-150 mg/kg. All animals were sacrificed under ether anaesthesia 2-3 hours after the last injection. Cats received: a) nialamide 100 mg/kg, i.p., alone, or b) nialamide 50-100 mg/kg followed 1 h later by L-tryptophan 25-50 mg/kg. Cats were killed by decapitation under barbiturate anaesthesia 1 h after the last injection.

After decapitation, brains were rapidly dissected out, blocks of the tissue to be investigated rapidly frozen in liquid propane cooled by liquid nitrogen, freeze-dried and processed for Falck-Hillarp fluorescence histochemical detection of monoamines (see Björklund et al. 1972). To achieve high fluorescence yield for



serotonin, a two-step formaldehyde treatment was often used: 1 h at 80°C with paraformaldehyde equilibrated at 50-70% relative humidity + 1 h at 80°C with paraformaldehyde equilibrated at 70-90% relative humidity. Following paraffin embedding, the specimens were sectioned and mounted for fluorescence microscopy. In most investigations alternate sections were stained with cresyl violet.

The glyoxylic acid method was used in the investigation of catecholaminergic innervation of rat inferior olive (paper II). This method involves perfusion with glyoxylic acid solution, Vibratome<sup>R</sup> sectioning, immersion in glyoxylic acid, and development at 100°C (for details see Lindvall and Björklund, 1974).

Microspectrofluorimetric analysis of formaldehyde induced fluorescence in neuronal cell bodies was performed with a modified Leitz microspectrograph. The recorded emission and excitation spectra were corrected according to standard procedures and expressed as relative quanta vs wavelength. (For detailed technical account, see Björklund et al. 1972, 1975).

#### CONVENTIONAL LIGHT MICROSCOPY

For cytoarchitectonic investigation of the brain stem (paper X), cats were anaesthetized with barbiturate, and fixed by perfusion through the heart with 4% paraformaldehyde in 0.1M phosphate buffer, pH = 7.4. Brains were left in fixative for six days, dehydrated in alcohol, and embedded in paraffin. Sections were stained with cresyl violet.

For histochemical investigation of secretory activity (paper IV), blocks comprising the subcommissural organ of rats were fixed for 24-72 h in Bouin's fixative. Paraffin sections of 3 µm thickness were cut, and stained with toluidin blue, periodic acid-Schiff (PAS), Bock's gallocyanine chromalum method for "neurosecretory substance", or the reaction for cystein (PFA-AB) (Pearse, 1968).





## ELECTRON MICROSCOPY

Rats were fixed by perfusion via the heart of a buffered solution of glutaraldehyde and paraformaldehyde. The composition of the fixative was either 2.5% glutaraldehyde + 1% paraformaldehyde in 0.1 M cacodylate buffer (pH = 7.4), or, 1% glutaraldehyde + 1% paraformaldehyde in 0.12 M phosphate buffer with 0.02 M  $\text{CaCl}_2$  (pH = 7.4). Blocks comprising the region to be investigated were cut out, rinsed in buffer, postfixated in 2%  $\text{OsO}_4$  for 2-4 h at 4°C, blockstained with uranyl acetate, dehydrated in ethanol, and embedded in Epon. With glass knives, semithin sections were cut for toluidin blue staining and light microscopical orientation. Ultrathin sections were cut with glass knives (occasionally, diamond knife), picked up on copper grids (or deposited on celloidin coated glass slides for radioautography), and contrasted with uranyl acetate and lead citrate.

## RADIOAUTOGRAPHY

For radioautographic visualization of serotonergic structures, rats were pretreated with monoamine oxidase inhibitor, and slowly infused with 200  $\mu\text{l}$   $10^{-4}$  M solution of tritiated serotonin ( $^3\text{H}$ -5-HI) via the intraventricular or intracisternal routes over 3 h. Cats received an intratissular injection of tritiated serotonin at a concentration of  $10^{-2}$  M. A total volume of 0.5  $\mu\text{l}$  was delivered over 3 h in 10 aliquots of 0.05  $\mu\text{l}$  using a glass micropipette inserted in the reticular formation a small distance (0.5-1.0 or 3 mm) from the locus coeruleus complex. In some cases, cold noradrenaline was added at a concentration 10 times higher than that of the tracer. Immediately after tracer infusion, the animals were fixed by perfusion of buffered glutaraldehyde-paraformaldehyde (see, "Electron microscopy"). Tissue was subsequently processed for electron microscopy-plastic or light microscopy-paraffin embedding. For light microscopic radioautography, series of 1  $\mu\text{m}$  thick plastic sections or 5  $\mu\text{m}$  thick paraffin sections were cut and mounted on glass slides. These were coated in Ilford K-5 emulsion diluted 1:1, exposed for 2, 15 and 30 days, developed with D-19 (Kodak), and



stained with toluidin blue or thionine-cresyl violet, respectively.

For electron microscopy, ribbons of pale gold sections were deposited on celloidin coated glass slides, stained with uranyl acetate and lead citrate, lightly vaporized with carbon, and coated with Ilford L-4 emulsion diluted 1:4. After 3, 6 or 12 months exposure these radioautographs were developed in para-phenylene diamine (Caro and Tubergen, 1962). The developed sections were picked up on naked copper grids, and, after thinning the celloidin film with isoamylacetate (Larra and Droz, 1970), examined in the electron microscope.

#### QUANTITATIVE MICROSCOPICAL DETERMINATIONS

The number of serotonergic cells in different regions was determined in the fluorescence microscope by counting on frontal sections, correcting according to Abercrombie (1946) and multiplying with the sampling interval. The simpler method of counting only cell bodies demonstrating a nucleolus was used when quantifying neuronal populations in conventional light microscopy.

Serotonin nerve terminal densities were determined by plotting radioautographically labelled sites in 1  $\mu$ m thick plastic sections with camera lucida technique. Terminal numbers were counted manually, and corrected according to Abercrombie's formula. Investigated nuclear areas were determined by cutting out the outlined nuclei in transparent paper, which was weighed. Multiplication with the known section thickness yielded tissue volume, and the terminal density was expressed as boutons/mm<sup>3</sup> of tissue.

#### BIOCHEMICAL DETERMINATIONS

<sup>3</sup>H-5-HT uptake measurements. Slices or homogenates of neuronal tissue were incubated in  $0.5 \times 10^{-7}$  M solution of <sup>3</sup>H-5-HT for 10 minutes, rinsed for 5 minutes, and dissolved in Soluene (Packard). Total accumulated tritium was measured by scintillation counting. Active uptake was calculated as uptake at 37°C minus uptake at 0°C. (For detailed account, see paper VIII).





Endogenous serotonin content was measured fluorimetrically according to Bertler (1961).

Metabolism of  $^3\text{H}$ -tryptophan. Slices of neuronal tissue were incubated in L- $^3\text{H}$ -tryptophan for 30 minutes, rinsed, and homogenized in acetic acid. After centrifugation, samples of the supernatant were spotted onto silica gel thin-layer plates. The chromatographs were run bidimensionally in ethylacetate-isopropanolol-ammonia (9:7:4, with 2 mg EDTA/100 ml) and acetic acid-ethylacetate (5:95), for efficient separation of the different metabolites formed. The serotonin and 5-hydroxy-indoleacetic acid spots were localized under UV light, scraped off, extracted and measured by liquid scintillation. In addition,  $^3\text{H}$ -5-hydroxyindoleacetic acid released into the incubation medium was determined. (For detailed technical account, see paper VIII).

#### PHARMACOLOGICALLY INDUCED TREMOR

Behavioral experiments. Under light ether anaesthesia rats received an intravenous injection of harmaline 5 mg/kg. They quickly awoke from anaesthesia, and the effects of harmaline were studied for an observation period of 45 minutes. Tremor was evaluated subjectively by two independent observers according to a 9-grade scale. The highest score during the 45 minutes observation period was taken as indicating the responsiveness to harmaline.

Electrophysiology. Silver-ball surface electrodes were placed in electrophysiologically identified longitudinal zones of cerebellar cortex. Spontaneous and harmaline induced activity was recorded with conventional amplifiers and oscilloscopes, and photographed on moving film or as superimposed sweeps. Mean frequency and amplitudes of harmaline induced mass climbing fiber activity was calculated.



## RESULTS AND COMMENTS

### A. DISTRIBUTION OF SEROTONINERGIC CELL BODIES

(Papers I, VII, X)

The early work of Dahlström and Fuxe (1964) provided a comprehensive description of the serotonergic nerve cell body distribution in rat brain stem. The cat is, however, also a widely used experimental animal in neuroanatomical as well as neurophysiological work. But for this species a detailed description of serotonergic cell body distribution has been lacking.

Large aggregations of noradrenergic cells are found in the cat dorsolateral pontine tegmentum within the locus coeruleus, subcoeruleus, medial and lateral parabrachial nuclei, and nucleus of Kölliker-Fuse. Collectively, these aggregations are referred to as the locus coeruleus complex. We have found that in addition to noradrenergic cells the locus coeruleus complex contains a considerable indolaminergic cell component (paper VII). Fluorescence microscopically, these cells displayed the same characteristics (yellow colour and fading under the short wave length illumination) as the known serotonin-containing cells of the raphe, and with microspectrofluorimetric technique it was shown that their spectral characteristics were identical. Radioautographic experiments demonstrated a corresponding group of serotonin accumulating neurons in the locus coeruleus complex. In other words, we were able to demonstrate two characteristic features of serotonergic neurons: endogenous serotonin content and uptake mechanisms. The serotonergic cells occurred in all subdivisions of the locus coeruleus complex, but they were most frequent in the medial parts of the locus coeruleus proper and subcoeruleus area.

The unexpected finding of serotonergic cells in the locus coeruleus complex gave the impetus for a general investigation of the monoamine cell distribution in cat brain stem (paper X). We found that the principles of serotonergic cell distribution are similar to those observed in the rat. Thus, the serotonergic cells were concentrated in raphe nuclei: nucleus raphe pallidus (RP), raphe obscurus (RO), raphe magnus (RM), raphe pontis (RPo), raphe centralis superior (RCS), raphe dorsalis (RD) and nucleus linearis intermedius (LI). However, large numbers of serotonergic cells were also found at all brain stem levels in areas outside the raphe nuclei. In the medulla oblongata, a band of scattered serotonergic cells extend laterally from the ventral RP to the position of the rubrospinal bundle at the lateral side of the





magnocellular lateral reticular nucleus. At the level of RM, a similar lateral extension was found overlying the superior olive and surrounding the facial nucleus. In pons, large numbers of serotonergic cells in periventricular gray and subjacent tegmentum form a lateral extension from the RD, dorsal RCS and RPo. Large numbers of serotonergic cells are also located in the lateral pontine tegmentum within the locus coeruleus (c.f. above), and in the vicinity of the motor trigeminal nucleus and exiting facial nerve. In mesencephalon, serotonergic cells were consistently observed within nc. interpeduncularis, n.c. interfascicularis, ventral tegmental area, the dorso-medial area of substantia nigra and scattered in the periventricular gray.

Quantitative estimations indicated that the total number of serotonergic cells in the cat brain stem is around 60 300. The majority of these (46 700 or 77.5%) are located within raphe nuclei. Investigation of Nissl stained material showed, however, that the serotonergic neurons are by far not the only cell component present in raphe nuclei. In addition, all raphe nuclei contain other types of medium-sized, presumably projecting neurons, and small-sized interneurons. The number of serotonergic cells in different raphe nuclei and the percentage these represent of the total number of medium-sized cells was: RD about 24 300 (70%), RP 8 000 (50%), RCS 7 400 (35%), RM 2 400 (15%), RO 2 300 (35%), LI 2 100 (25%), and RPo some 280 serotonergic cells (10%). Of the approximately 13 600 serotonergic cells outside the raphe nuclei, the locus coeruleus complex accounted for about 1 300, interpeduncular nucleus 1 400, the lateral extensions from ventral raphe (pons and medulla oblongata) for 2 800 and the lateral extensions from dorsal raphe (pons and mesencephalon) for about 5 200 cells.

The pineal gland of the rat is connected to the epithalamus by a long slender stalk, which ends as an aggregation of cells, the so-called lamina intercalaris, between the posterior and habenular commissures. The pineal cells, as well as cells in the stalk and lamina intercalaris contain serotonin, and receive a rich peripheral sympathetic innervation. Scattered yellow fluorescent cells and sympathetic innervation can also be found in the habenular nuclei and among the fibers of the stria medullaris. Björklund and co-workers (1973a) suggested that the small serotonin-containing cells found in the habenular region of the rat may be of neuronal nature. The study described in paper I was undertaken to test this hypothesis. The appearance of the cells in habenula was found similar to that of the cells in lamina intercalaris



and pineal stalk. A close relation to the sympathetic nerve fibers indicated that the serotonin-containing cells in the habenula are the target of this innervation. Further, a marked inter-individual variation in the distribution of sympathetic innervation and yellow fluorescent cells was observed. It is known that serotonergic nerve cell bodies acquire fluorescence histochemically detectable serotonin content during fetal life, but their axons grow out later and reach their target areas during the first postnatal week (Seiger and Olson, 1972; Olson and Seiger, 1973). Pinealocytes are known to develop fluorescence later (first two weeks after birth) (Håkanson et al., 1967). It was found that the serotonergic cells in habenular nuclei and stria medullaris develop (as expressed by serotonin content) at a very late stage (3 weeks - 2 months postnatally), and subsequent to the cells of pineal, pineal stalk and lamina intercalaris. In conclusion, it was suggested that the serotonergic cells in the habenular nuclei are of pinealocyte and not neuronal nature.



## B. SEROTONINERGIC INNERVATION OF THE INFERIOR OLIVE

(Papers II, III and XI)

The inferior olive is a nuclear complex located in the ventral medulla oblongata and consists of three major subnuclei: the principal olivary nucleus (PO), the medial accessory nucleus (MAO) and the dorsal accessory nucleus (DAO). The neurons of the inferior olive project to the cerebellum and are believed to be the unique source of the strongly excitatory climbing fibers to the Purkinje cells (Eccles et al., 1967; Desclin et al., 1974; Montarolo et al., 1979). Investigations in the cat have shown that the olivo-cerebellar climbing fiber projection is strictly topographically organized (Armstrong et al., 1974; Brodal and Walberg, 1977 a, b; Groenewegen and Voogd, 1977; Groenewegen et al., 1979; Kawamura and Hashikawa, 1979). Thus, distinct compartments of the inferior olive project to different narrow longitudinal zones of cerebellar cortex. The nomenclature of Voogd (1969) naming these zones A, B, C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, D<sub>1</sub> and D<sub>2</sub> is today generally accepted. Neurophysiological investigations have shown that spino-cerebellar climbing fiber paths terminate in similar longitudinal zones (Oscarsson and Sjölund, 1977a, b; Ekerot and Larson, 1980).

### 1. Serotonergic innervation

The serotonergic innervation of the cat and rat inferior olive was investigated with Falck-Hillarp fluorescence histochemical technique (Paper II). In the cat, a rich serotonergic innervation with a complex distribution was revealed. The topography of the serotonergic innervation agreed well with the topography of the efferent olivocerebellar projection (Fig. 12, paper II). The densest serotonergic innervation was found in the caudal DAO, which projects to the B zone of vermis. A dense plexus was also present in the caudal MAO, which is origin of climbing fiber innervation to the vermian A zone. A sparser plexus was observed in the rostro-medial parts of DAO, which project to the C<sub>1</sub> and C<sub>3</sub> zones of pars intermedia. No serotonergic innervation was detected in rostral MAO, which projects to the C<sub>2</sub> zone. A sparse serotonergic plexus was found in the ventral lamella of PO (it is debatable whether this part of PO projects to the D<sub>1</sub> or D<sub>2</sub> zone of the cerebellar hemisphere; Dietrichs, personal communication). In the rat inferior





olive serotonergic innervation could be detected only in the lateral part of DAO. It remains to be settled if the observed difference in serotonergic innervation of cat and rat inferior olive depends on true interspecies differences, or if the limited sensitivity of the Falck-Hillarp technique led to failure detecting serotonergic innervation in certain parts of the rat inferior olive.

The ultrastructure of the serotonergic innervation of the inferior olive has so far only been investigated in the lateral DAO of rat (Paper XI). Serotonin accumulating nerve terminals were visualized with radioautographic technique. The boutons of these fibers were marked by a content of microcanalicular (or microvesicular) organelles, larger tubular-vesicular elements, and large granular vesicles. The boutons were frequently observed in close apposition to dendritic shafts and spines, but few engaged in classical synaptic junctions. These observations indicate that the serotonergic innervation is of the "non-synaptic" variety (see General Introduction), and that the terminals exert their action directly onto the olivary neurons.

## 2. Harmaline induced tremor

Harmaline induced tremor is known to be generated in certain parts of the inferior olive (De Montigny and Lamarre, 1973; Llinas and Volkind, 1973; Lamarre and Puil, 1974). Administered systemically or locally, harmaline induces rhythmic, synchronous firing at around 8-12 Hz in large numbers of olivary neurons. This activity is transmitted to the cerebellum via climbing fibers, and reaches the spinal cord via efferent cerebellar pathways. The influence of this rhythmic activity on the spinal motoneurons results in tremor. We decided to investigate if the serotonergic innervation plays a role in this tremor phenomenon. Indeed, such a relationship would be in line with certain pharmacological information. Thus, harmaline is a potent inhibitor of monoamine oxidase A (e.g. Fuller, 1972; Buckholtz and Boggan, 1977a), and also inhibits neuronal serotonin uptake (Buckholtz and Boggan, 1977b).

The results of De Montigny and Lamarre (1973) recording harmaline induced activity in the cat inferior olive indicates that harmaline exerts its tremogenic effect in those parts of MAO and DAO, which receive dense serotonergic innervation (see their Fig. 3). This possible topographic relation between serotonergic innervation and harmaline induced rhythmic activity was further explored by us (Paper III). Surface electrodes were placed in the electrophysiologically identified



a, b<sub>2</sub>, c<sub>1</sub>, c<sub>2</sub> and c<sub>3</sub> zones (considered analogous to the anatomically defined A, B, C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub> zones) of cerebellar cortex (lobules IV and V). With this technique mass Purkinje cell activity can be recorded, and the amplitude of the recorded potentials is proportional to the number of Purkinje cells activated synchronously. Administration of harmaline (5 mg/kg, i.v.) resulted in appearance of rhythmic mass Purkinje cell activity with the characteristics of climbing fiber evoked activity. The synchronous activity often appeared in groups with an internal frequency of 10-12 Hz, but were separated by silent periods. (Similar waxing and waning of harmaline induced activity was also noticed in the recordings directly from the olive by De Montigny and Lamarre (1973), and may depend on variations in excitability of olivary neurons). The mean frequency and amplitude of harmaline induced activity in each zone was calculated from moving film recordings of periods of maximal drug effect. Both these parameters indicated a close relationship between harmaline induced activity and distribution of serotonergic innervation. Thus, the strongest rhythmic activity was recorded on the b<sub>2</sub> zone, which receives climbing fibers from the densely innervated caudal parts of DAO. Strong activity was recorded in the a zone, which receives input from the well innervated caudal parts of MAO. In pars intermedia, the c<sub>1</sub> and c<sub>3</sub> zones showed more rhythmic activity than the c<sub>2</sub> zone, which parallels the observation of more serotonergic innervation in rostro-medial DAO than rostral MAO.

In the rat, destruction of the serotonergic innervation by intraventricular injection of 5,6-DHT or 5,7-DHT was found to decrease both the electrophysiologically recorded mass climbing fiber activity in cerebellar cortex and the subjectively estimated tremor induced by harmaline. Taken together, the observations in paper III indicate that harmaline may act through the serotonergic innervation.

### 3. Serotonin syndrome

Pharmacologically, a "serotonergic motor syndrome" can be elicited in the rat by administration of serotonin receptor agonists, serotonin releasing drugs, or monoamine oxidase inhibition + L-tryptophan loading (which is believed to result in overflow of transmitter from serotonergic nerve terminals) (Green and Grahame-Smith,





1976; Jacobs, 1976). One component of this syndrome is tremor, while other expressions include forepaw-treading, lateral head waving, hind-limb abduction and Straubs's tail. We have in a series of experiments found evidence that the tremor component of this serotonin syndrome is generated in the inferior olive (preliminary communication; Wiklund et al., 1978, 1980; Sjölund et al., 1980).

With electrophysiological technique it was found that systemic administration of a serotonin receptor agonist (5-methoxy-N,N-dimethyltryptamine, 5 mg/kg i.v.) or monoamine oxidase inhibition (Pargyline 50 mg/kg, or Clorgyline 50 mg/kg i.p.) + L-tryptophan loading (150 mg/kg i.p.) leads to strong rhythmic, synchronous climbing fiber evoked activity in the cerebellum.

Tremor can be recorded by attaching a small magnet to the head of the rat, which is placed in an induction coil. The potential variations induced by the oscillatory movements of tremor are amplified and recorded (Dill et al., 1968). Using this technique we found that we could detect strong tremor after harmaline (10 mg/kg, i.p.) as well as Clorgyline (50 mg/kg i.p.) + L-tryptophan (150 mg/kg i.p.). In rats where most or all of the inferior olivary neurons had been destroyed by 3-acetylpyridine (Desclin and Escubi, 1974; Llinás et al., 1975), however, only weak or no tremor at all could be recorded. Together, these results indicate that (over-)stimulation of serotonergic receptors in the olive elicits the rhythmic, synchronous firing, which is expressed as tremor.

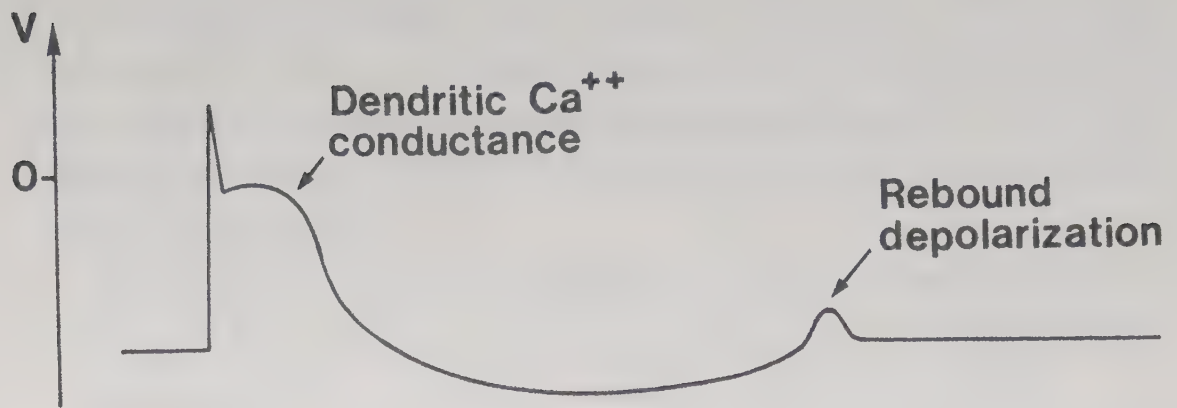
The major motor symptoms of the serotonin syndrome is, however, not generated in the olive. With an Animex II instrument generously provided by LKB Farad AB, Stockholm) we found that we could estimate the amount of hyperactivity, forepaw-treading etc, but that the conductance changes induced by the tremor of the serotonin syndrome was below the sensitivity of the instrument. After destruction of the inferior olive by 3-acetylpyridine, the non-tremor motor components of the serotonin syndrome remained.

#### 4. Neuromodulatory role of serotonin in inferior olive

The results described above indicate that the serotonergic innervation and receptors are critically involved in eliciting synchronous, rhythmic firing of olivary neurons. On the basis of various electrophysiological observations it is possible to formulate a hypothesis for a neuromodulatory action of serotonin on olivary neurons (Fig. 1), which can explain the tremogenic phenomena.



## OLIVARY ACTION POTENTIAL



## NEUROMODULATORY EFFECT OF SEROTONIN

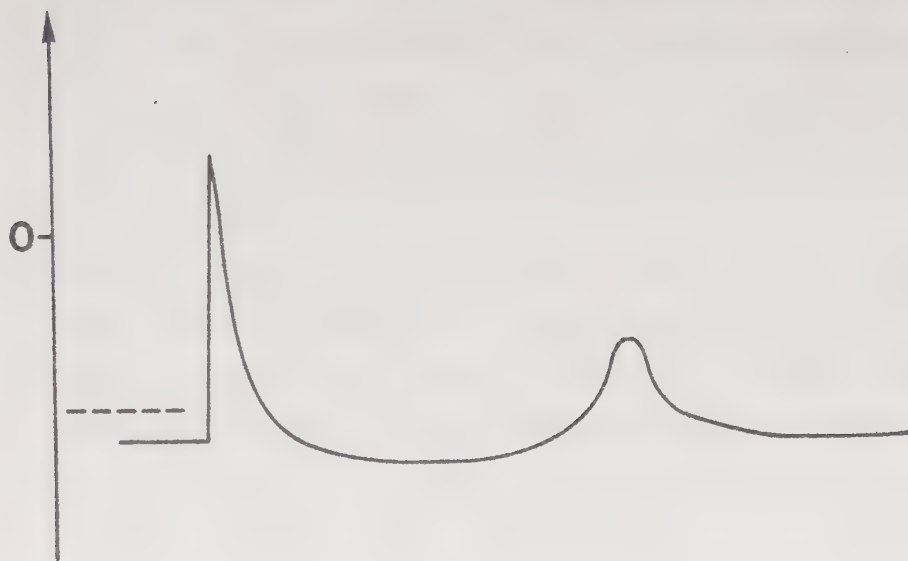


Fig 1

Proposed neuromodulatory effect of serotonin on olivary action potential. For description, see text.

11-11-11  
11-11-11  
11-11-11

11-11-11 11-11-11 11-11-11 11-11-11 11-11-11

11-11-11 11-11-11 11-11-11 11-11-11 11-11-11

In all species investigated, which include cat (Sotelo et al., 1974) and rat (Gwyn et al., 1977), olivary neurons have been shown to possess dendro-dendritic gap junctions, which result in electrotonic coupling (Llinás et al., 1974). It is generally agreed that these gap junctions must be the basis for the synchrony of olivary induced activity.

Olivary neurons demonstrate a complex action potential consisting of a fast initial spike, followed by a large delayed depolarization and a long hyperpolarization, which is terminated by a rebound potential (Crill, 1970; Llinas and Yarom, 1980). Llinas and Yarom (1980) have investigated the ionic mechanisms underlying these different components. The fast initial spike is a classical sodium conductance, but the delayed depolarization corresponds to a dendritic calcium conductance. Since an increase in intracellular  $\text{Ca}^{++}$  concentration is known to block conductivity over gap junctions (Rose and Loewenstein, 1976; Loewenstein and Rose, 1978; Baux et al., 1978) we suggest that one effect of the dendritic  $\text{Ca}^{++}$  potential is a transient decrease in electrotonic coupling. The rebound potential constitutes a rapidly inactivating somatic  $\text{Ca}^{++}$  conductance (Llinás and Yarom, 1980). Interestingly, Llinás and Yarom (1980) noted that a general hyperpolarization of the olivary neuron led to a marked augmentation and earlier occurrence of the rebound potential in the complex action potential. They pointed out, that not only did the augmented rebound potentials lead to facilitation, but could even elicit new action potentials and oscillatory activity in the neuron.

Headley and Lodge (1976) investigated some effects of iontophoretic application of serotonin on olivary cells. Interestingly, they recorded two effects. First, serotonin suppressed the delayed depolarization now known to be a dendritic  $\text{Ca}^{++}$  conductance. According to our hypothesis this would mean that the gap junctions would be kept open and the olivary cells tend to synchronize their activity. Second, serotonin seemed to have a hyperpolarizing effect on the olivary neurons (as indicated by an increased height of the initial fast spike). According to Llinás and Yarom (1980) this would lead to augmentation of the rebound potential and favour the development of oscillatory activity. In conclusion, serotonin could account for both the synchrony and rhythmicity of the olivary induced activity, which has been shown to be the basis for the observed tremor phenomena.





## C. SEROTONINERGIC INNERVATION OF THE RAT SUBCOMMISSURAL ORGAN. (Papers IV and V)

The subcommissural organ (SCO) is a specialized area of ependyma attached to the ventral surface of the posterior commissure, where it forms the lining of the roof of the cerebral aqueduct at its junction with the third ventricle. The secretory activity of SCO is well established (for references, see paper IV). Secretory material of glycoprotein nature is released into the ventricular fluid where it forms the so-called Reissner's fiber, and in some species there is evidence of a basal secretion into a vascular plexus underlying the organ. The physiological significance of these secretions is, however, obscure.

Fluorescence histochemically, a serotonergic innervation of the basal SCO has only been described in the rat and gerbil (Fuxe et al., 1968; Wiklund et al., 1977) while the SCO of mouse, rabbit and cat (Wiklund et al., 1977; unpublished results) seems to lack such innervation. In the studies described in papers IV and V, we investigated the ultrastructure of the neuronal input to the rat SCO. In the electron microscope, numerous thin unmyelinated varicose axons were found in the basal SCO. The boutons of these fibers contained many small clear round-elongated synaptic vesicles, a few large granular vesicles and mitochondria. The boutons were frequently seen to engage in one or several assymetric synapses with the specialized SCO cells. In order to determine if the ultrastructurally observed innervation was identical to the fluorescence histochemically detected serotonergic innervation, rats were administrated 5,6-DHT or 5,7 DHT via the intraventricular route. One, two and three days after this treatment virtually all boutons contacting SCO cells demonstrated different stages of degeneration. We concluded that the ultrastrucutally observed boutons were identical to the serotonergic innervation, and that this formed the major neuronal input to the rat SCO. Independently of us Bouchaud and Arluison (1977) reached the same results with radioautographic techniques.

In the SCO cells neurotoxic destruction of the serotonergic input led to pronounced cytological changes. Ultrastructurally, there was an enormous swelling of the endoplasmic reticulum containing floccular material, increase in Golgi complex size, accumulation of secretory granules in apical and basal SCO cell processes, and evidences of increased release of secretory material. These changes



24

developed within the first days after 5,6-DHT and 5,7-DHT, and were still present after 8 months. Histochemically, increased reactions for neurophysin-like material and glycoproteins were detected 1-4 days after neurotoxin administration. Further, pronounced increases in size of nuclei and prominence of nucleoli were noted light microscopically. We interpreted these effects as responses to the serotonergic denervation, and suggested that serotonin could exert a powerful inhibitory influence on the synthetic and secretory activities of the SCO. However, in view of recent reports of non-serotonergic innervation of SCO (Bouchaud, 1979) and GABA accumulating nerve fibers in the SCO (Belin, personal communication) this conclusion should be corroborated by additional experiments. Obvious experiments would be to see if electrolytic lesioning of raphe nuclei produce the same effects as neurotoxic denervation, and trying to counteract the denervation induced secretory increase in SCO by ventricular infusion of serotonin.





#### D. PLASTICITY OF SEROTONINERGIC CONNECTIONS AFTER NEUROTOXIC LESIONS (Papers V, VI, VIII, IX and XI)

Very limited and unsuccessful regeneration is usually seen after transections of mammalian central nervous connections. The failure of regeneration was by Windle and co-workers considered to depend on glial scar formation at the site of mechanical transection interfering with penetration of the sprouting axons (Windle, 1955; Clemente 1964). Observations of plastic remodelling of the septal neuropil after fimbrial and medial forebrain bundle lesions led Raisman (Raisman, 1969; Raisman and Field, 1973) to formulate an alternative hypothesis. According to this hypothesis, intact afferents will react by sprouting to occupy the synaptic sites vacated by the lesion, and this deprives the lesioned axons of some important growth stimulus. Experiments on serotonergic systems may provide further insight into the factors limiting central nervous regeneration. With the neurotoxic drugs 5,6-DHT and 5,7-DHT it is possible to lesion serotonergic axons selectively without formation of glial scars. A number of previous biochemical and fluorescence histochemical investigations have, indeed, demonstrated that after this kind of lesioning the severed serotonergic neurons are able to regrow and reform some of their connections (e.g. Björklund et al. 1973b; Nobin et al., 1973; Nygren et al., 1974).

##### 1. Mode of termination and course of neuroplasticity.

In many areas of the brain it has been shown that the serotonergic terminals form very few classical synaptic junctions. Such "non-synaptic" serotonergic innervation has been observed in cerebral cortex (Descarries et al., 1975), caudate nucleus (Calas et al., 1976; Arluison and De La Manche, 1980), and locus coeruleus (Léger and Descarries, 1978). "Synaptic" serotonergic innervation, where each bouton forms at least one synapse, has, however, been described in some areas. These include the subcommissural organ (Bouchaud and Arluison, 1977; paper V) and the suprachiasmatic nucleus (Nojyo and Sano 1978). In view of Raisman's hypothesis it seemed pertinent to investigate if these different modes of termination influence the plastic processes following neurotoxic axotomy.

In the study described in paper XI, we found with radioautographic technique that the serotonergic innervation of the rat lateral DAO is of the "non-synaptic"



variety. Only 5% of the profiles of serotonergic boutons demonstrated a synaptic junction. Geometrically, we estimated that with this frequency not more than one out of 7-8 boutons engage in a classical synapse. Administration of 5,6-DHT (75 µg, intraventricularly) induced an efficient (90%) acute denervation of the lateral DAO. Regeneration was rapid, however, and after 2 months a serotonergic plexus of normal density had been reformed.

The subcommissural organ, which receives a "synaptic" serotonergic innervation, was efficiently denervated by administration of 5,6-DHT or 5,7-DHT (paper V). Fluorescence histochemically, the subcommissural organ was studied for survival times of up to 15 months, but no signs of regeneration could be ascertained. However, when specimens from animals surviving for 1, 3 or 8 months were investigated electron microscopically, it was found that the subcommissural organ had become reinnervated by non-serotonergic nerve fibers. Our conclusion was that these foreign nerve fibers had replaced the serotonergic terminals lost by the lesion.

Based on these observations, we hypothesize that the mode of termination has very important bearings on the course of neuroplasticity following neurotoxic injury; "non-synaptic" connections will be reformed, while "synaptic" serotonergic connections are replaced by sprouting of intact afferents. Further support for this hypothesis can be found in the literature. In an investigation still in progress, we have found that the "non-synaptic" serotonergic innervation of locus coeruleus is regenerated after neurotoxic lesioning (prel. communication, Degueurce et al. 1979). The "synaptic" serotonergic innervation of the suprachiasmatic nucleus (Nojyo and Sano 1978) is, however, not regenerated (Björklund et al. 1973b; Wiklund unpublished). The proposed hypothesis is in line with the common conviction that competition for synaptic sites must play a role in the establishment of neuronal connections. It further implies that the "non-synaptic" serotonergic connections would be exempt from such competition. This condition would clearly favour a dynamic mode of function involving constant reshaping of these neuronal plexa.





## 2. Observations on the regeneration of the bulbospinal serotonergic system.

If the hypothesis presented above is correct, the regeneration after neurotoxic injury represents reformation of "non-synaptic" serotonergic connections. With biochemical and fluorescence histochemical techniques, we have investigated the regeneration observed in the bulbospinal system after lesioning with 75  $\mu$ g 5, 6-DHT, intraventricularly.

Biochemically (paper VIII) a drastic reduction of [ $^3$ H] 5-HT uptake, serotonin content, and conversion of [ $^3$ H] tryptophan into serotonin and 5-hydroxyindoleacetic acid was found in the spinal cord 1-2 weeks after 5, 6-DHT. The magnitude of decrease indicated a virtually total denervation of spinal tissue. A gradual recovery of [ $^3$ H] 5-HT uptake indicated progressive proximo-distal growth of the regenerating serotonergic axons. However, even after survival times of 7-8 months only subnormal levels were regained at all spinal levels. It was concluded that the regenerating serotonergic axons reach, but fail to restore normal density in the distant spinal target areas.

The fluorescence histochemical investigation (paper IX) was concentrated on the degenerative and regenerative phenomena in the medulla oblongata. Acutely, 1-3 weeks after 5, 6-DHT, extensive denervation was noted in many areas, including spinal gray, inferior olive and facial nucleus. Large numbers of swollen distorted fibers appeared and were interpreted as the proximal stumps of the lesioned serotonergic axons. The distribution of these demonstrated that the bulbospinal axons had been severed at a high level, i.e. in caudal medulla oblongata and rostral-most cervical spinal cord. The first signs of regenerative sprouting from these proximal stumps appeared already one week after 5, 6-DHT. The rate of reinnervation of different target areas depended on the distance to the regrowing axons. It was particularly rapid in the inferior olive, where some of the sprouting actually took place directly into the denervated neuropil. The regrowth process demonstrated a high degree of precision. Thus, serotonergic plexa were reestablished in the inferior olive, facial nucleus and other regions, which normally received serotonergic innervation, but surrounding tissue was not invaded. This specificity indicates that the regenerative growth process is determined by mechanisms of guidance and recognition, which presumably are similar, or identical, to those regulating ontogenetic development of the serotonergic system. Limitations to these regulatory mechanisms were, however, demonstrated. First, there was a strikingly abnormal proximo-distal distribution of





regenerated terminals. Distal terminal areas in spinal gray regained only a small part of their normal terminal density, while abnormal hyperinnervations developed in proximal target areas of the brain stem. Further, in the inferior olive, regrowth was not limited to those parts where the normal serotonergic innervation is confined. Instead, the regrowing fibers invaded and established dense plexa in many other parts of the olivary complex. Presumably, these abnormalities in the distribution of regenerated terminals reflect the perturbations imposed by regrowth in the adult as opposed to the immature developmental situation. By analysis of these phenomena, we may therefore gain some insight into the principles of the mechanisms regulating establishment of these serotonergic connections. Hence, we suggested that the drive of axonal growth is derived from an inherent program of the serotonergic neurons to establish a predetermined amount of axonal arborizations. The role of the target tissue may be a mere provision of cues for recognition, rather than a truly neurotrophic influence. The regulatory neuronal program obviously lacks a mechanism to terminate further proliferation when an innervation of normal density has been reformed. To result in the intended distribution of terminals it is conceivable that the growth must be precisely timed in relation to the general growth and development of the nervous system.



## PERSPECTIVES

A great amount of information has accumulated on the central serotonergic systems since Twarog and Page in 1953 first detected this substance in the brain. Still, it is obvious that much more work is needed before we can gain deeper insight into how these neurons interact with other cellular elements of the brain. I would only wish to point out some of the most obvious questions that have come to my mind in the course of this work.

Much of our anatomical information on the efferent connections of raphe neurons is based on studies that failed to distinguish between serotonergic and non-serotonergic components. Differential studies are clearly needed.

Much more information is needed on how the serotonergic neurons interact with their target neurons. The classical question if the serotonergic input is excitatory or inhibitory, and the technique of spontaneous spike frequency analysis may in most instances be inadequate. The ionic mechanisms mediating the serotonergic response must be investigated. The newly developed techniques of intracellular recordings in vitro opens possibilities to tackle these questions. However, the serotonergic systems may also influence "biochemical" aspects of target cell activity, why the investigations must not be limited to electrophysiological techniques.

Except for some retrograde horseradish peroxidase studies, little is known about the afferent inputs to raphe nuclei. Hopefully, combined anatomical, ultrastructural and electrophysiological approaches will demonstrate how the afferents are integrated and interact with the serotonergic and non-serotonergic neurons.

The plastic properties demonstrated by serotonergic neurons could tell us much of how the central nervous system is developed and maintained. The variety of termination properties and neuron-target interaction provides possibilities to investigate many aspects of neuronal connectivity. The hypothesis that serotonergic innervation has a plastic mode of function involving constant reshaping of terminal plexa should, if possible, be put to testing.





## ACKNOWLEDGEMENTS

I am indebted to a large number of people for their support in the presently reported thesis work. I wish to take this opportunity to extend my sincere thanks to them.

I thank Profs. Bengt Falck and Christer Owman, heads of the Department of Histology during the years of my thesis work, for their continuous friendly support.

Dr. Anders Björklund introduced me into these exciting fields of research, and has served as supervisor of my work. It is my hope that we will continue to enjoy fruitful collaboration and exchange for many years to come.

The scientific and personal support of Dr. Kjeld Møllgård has been of very special importance for me.

I thank Prof. Olov Oscarsson and the whole Department of Neurophysiology for allowing me to become part of their group and opening my eyes to the fascinating field of electrophysiology.

I warmly thank all those I have had the privilege to collaborate with, including Lucienne Léger, Yael Balslev, Bo Bjerre, Amanda Degueurce, Laurent Descarries, Ingemar Lorén, Jesper Lundberg, Kjeld Møllgård, Mats Persson and Bengt Sjölund.

Many people have opened their laboratories and taught me new techniques. Drs. Geoffrey Raisman, Pauline Field, and Anna Östberg (Oxford and London) taught me quantitative electron microscopy of central nervous tissue. Prof. Laurent Descarries, Kenneth C. Watkins, and Silvia Garcia (Montréal) have instructed me in the radioautographic visualization of monoaminergic neurons at light and electron microscopical levels. I have very much appreciated the continuous possibility to work at the Anatomy Department A at the University of Copenhagen. Dr. Jean-Francois Pujol and Prof. Michel Jouvet have given me the possibility to work for several inspiring periods at the Département de Médecine Expérimentale, Université Claude Bernard, Lyon, and I thank Prof. Jan Voogd and his collaborators for the very enjoyable month I spent at the Laboratory of Anatomy and Embryology, University of Leiden.

I am greatly indebted to Siv Carlsson, Birgitta Fogelström, Kerstin Fogelström, Birgit Haraldsson, Anna Holender, Sten Håkansson, Ulla Jarl, Carlota Johnsen, Britt Lernius, Britt Lindberg, Gunilla Lundqvist, Eva Moghimzadeh, Gertrude



Stridsberg, and Ella Sundström for all they have put into the work leading to this thesis. It is my sincere hope they realize that the results are theirs as much as mine.

The quality of photographs and other illustrations are credited to Krister Nilsson, Hjalmar Gustavsson, Keld Ottesen, Agneta Persson and Lasse Sandström.



## REFERENCES

- Abercrombie, M. Estimation of nuclear population from microtome sections. *Anat. Rec.* 94 (1946) 239-247.
- Aghajanian, G.K. and R.Y. Wang. Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique. *Brain Res.* 122 (1977) 229-242.
- Amin, A.H., T.B.B. Crawford and J.H. Gaddum. The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol.* (London) 126 (1954) 596-618.
- Andén, N.-E., A. Dahlström, K. Fuxe, K. Larsson, L. Olson and U. Ungerstedt. Ascending monoamine neurons to the telencephalon and diencephalon. *Acta physiol. scand.* 67 (1966) 313-326.
- Andén, N.-E., M.G.M. Jukes and A. Lundberg. Spinal reflexes and monoamine liberation. *Nature* 202 (1964) 1222-1223.
- Arluison, M. and I.S. De La Manche. High-resolution radioautographic study of the serotonin innervation of the rat corpus striatum after intraventricular administration of [ $^3\text{H}$ ] 5-hydroxytryptamine. *Neuroscience* 5 (1980) 229-240.
- Armstrong, D.M., R.J. Harvey and R.F. Schild. Topographical localization in the olivo-cerebellar projection: an electrophysiological study in the cat. *J. Comp. Neurol.* 154 (1974) 287-302.
- Azmitia, E.C. and M. Segal. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179 (1978) 641-668.
- Barasi, S. and M.T.H. Roberts. The modification of lumbar motoneurone excitability by stimulation of a putative 5-hydroxytryptamine pathway. *Br. J. Pharmacol.* 52 (1974) 339-348.
- Basbaum, A.I., C.A. Clanton and H.L. Fields. Three bulbospinal pathways from the rostral medulla of the cat: An autoradiographic study of pain modulating systems. *J. Comp. Neurol.* 178 (1978) 209-224.





- Basbaum, A.I. and H.L. Fields. The origin of descending pathways of the spinal cord of the cat and rat: Further studies on the anatomy of pain modulation. *J. Comp. Neurol.* 187 (1979) 513-532.
- Baumgarten, H.G., A. Björklund and W. Wuttke. Neural control of pituitary LH, FSH and prolactin secretion: the role of serotonin. In D.E. Scott, G.P. Kozlowski and A. Weinl (Eds.), Brain Endocrine Interaction III. Neural Hormones and Reproduction, S. Karger AG, Basel, 1978, pp. 327-343.
- Baumgarten, H.G., L. Lachenmayer and A. Björklund. Chemical lesioning of indole-amine pathways. In R.D. Myers (Ed.), Methods in Psychobiology, Vol. 3, Academic Press, New York, 1977, pp. 47-98.
- Baux, G., M. Simmonne, L. Tauc and J.P. Segundo. Uncoupling of electronic synapses by calcium. *Proc. Nat. Acad. Sci. (USA)* 75 (1978) 4577-4581.
- Beaudet, A. and L. Descarries. The monoamine innervation of rat cerebral cortex: synaptic and nonsynaptic axon terminals. *Neuroscience* 3 (1978) 851-860.
- Bertler, Å. Effect of reserpine on the storage of catecholamines in brain and other tissues. *Acta physiol. scand.* 51 (1961) 75-83.
- Björklund, A., B. Falck and O. Lindvall. Microspectrofluorimetric analysis of cellular monoamines after formaldehyde or glyoxylic acid condensation. In P.B. Bradley (Ed.), Methods in Brain Research, John Wiley, London, 1975, pp. 249-294.
- Björklund, A., B. Falck and Ch. Owman. Fluorescence microscopic and microspectrofluorimetric techniques for the cellular localization and characterization of biogenic amines. In J.E. Rall and I.J. Kopin (Eds.), Methods in Investigative and Diagnostic Endocrinology, Vol. 1, The Thyroid and Biogenic Amines, Elsevier North Holland, Amsterdam, 1972, pp. 318-368.
- Björklund, A., A. Nobin and U. Stenevi. The use of dihydroxytryptamine as tools for morphological studies and localized lesioning of central indolamine neurons. *Z. Zellforsch.* 145 (1973a) 470-501.
- Björklund, A., A. Nobin and U. Stenevi. Regeneration of central serotonin neurons after axonal degeneration induced by 5,6-dihydroxytryptamine. *Brain Res.* 50 (1973b) 214-220.
- Boakes, R.J., P.B. Bradley, I. Briggs and A. Dray. Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: A possible neuronal basis for the actions of LSD 25. *Br. J. Pharmacol.* 40 (1970) 202-218.



- Bobillier, P., S. Seguin, A. Degueurce, B.D. Lewis and J.F. Pujol. The efferent connections of the nucleus raphe centralis superior in the rat as revealed by radioautography. *Brain Res.* 166 (1979) 1-8.
- Bobillier, P., S. Seguin, F. Petitjean, D. Salvert, M. Touret and M. Jouvet. The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res.* 113 (1976) 449-486.
- Brodal, A. and F. Walberg. The olivocerebellar projection in the cat studied with the method of retrograde axonal transport of horseradish peroxidase. IV. The projection to the anterior lobe. *J. Comp. Neurol.* 172 (1977a) 85-108.
- Brodal, A. and F. Walberg. The olivocerebellar projection in the cat studied with the method of retrograde axonal transport of horseradish peroxidase. VI. Projection onto longitudinal zones of the paramedian lobule. *J. Comp. Neurol.* 176 (1977b) 281-294.
- Bouchaud, C. Evidence for a multiple innervation of subcommissural ependymocytes in the rat. *Neurosci. Lett.* 12 (1979) 253-258.
- Bouchaud, C. and M. Arluison. Serotonergic innervation of ependymal cells in the rat subcommissural organ. A fluorescence, electron microscopic and radioautographic study. *Biologie Cellulaire* 30 (1977) 61-64.
- Buckholtz, N.S. and W.O. Boggan. Monoamine oxidase inhibition in brain and liver produced by  $\beta$ -carbolines: structure-activity relationships and substrate specificity. *Biochem. Pharmacol.* 26 (1977a) 1991-1996.
- Buckholtz, N.S. and W.O. Boggan. Inhibition by  $\beta$ -carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. *Life Sci.* 20 (1977b) 2093-2100.
- Calas, A., M.J. Besson, C. Gaughy, G. Alonso, J. Glowinski and A. Cheramy. Radioautographic study of in vivo incorporation of  $^3\text{H}$ -monoamines in the cat caudate nucleus: identification of serotonergic fibers. *Brain Res.* 118 (1976) 1-13.
- Caro, L.G. and R.P. van Tubergen. High resolution autoradiography. I. Methods. *J. Cell Biol.* 15 (1962) 173-188.
- Chan-Palay, V. Fine structure of labelled axons in the cerebellar cortex and nuclei of rodents and primates after intraventricular infusions with tritiated serotonin. *Anat. Embryol.* 148 (1975) 235-265.





Clemente, C.D. Regeneration in the vertebrate central nervous system. *Int. Rev. Neurobiol.* 6 (1964) 257-301.

Crill, W.E. Unitary multiple-spiked responses in cat inferior olive nucleus. *J. Neurophysiol.* 33 (1970) 199-209.

Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* 62, suppl. 232 (1964).

Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. *Acta physiol. scand.* 64, suppl. 247 (1965) 5-36.

Degueurce, A., L. Wiklund, L. Léger and J. F. Pujol. Evidences of functional serotonergic reinnervation in rat locus coeruleus following 5,6-DHT and 5,7-DHT induced denervation. *Neurosci. Lett., Suppl.* 3 (1979) S 365.

De Montigny, C. and Y. Lamarre. Rhythmic activity induced by harmaline in the olivo-cerebello-bulbar system of the cat. *Brain Res.* 53 (1973) 81-95.

Descarries, L., A. Beaudet and K.C. Watkins. Serotonin nerve terminals in adult rat neocortex. *Brain Res.* 100 (1975) 563-588.

Desclin, J.C. Histological evidence supporting the inferior olive as the major source of cerebellar climbing fibers in the rat. *Brain Res.* 77 (1974) 365-384.

Desclin, J.C. and J. Escubi. Effects of 3-acetylpyridine on the central nervous system of the rat, as demonstrated by silver methods. *Brain Res.* 77 (1974) 349-364.

Dill, R.E., H.L. Dorman and W.M. Nickey. A simple method for recording tremors in small animals. *J. Appl. Physiol.* 24 (1968) 598-599.

Eccles, J.C., M. Ito and J. Szentágothai. The Cerebellum as a Neuronal Machine. Springer, New York, 1967.

Ekerot, C.F. and B. Larson. The dorsal spino-olivocerebellar system in the cat. I. Functional organization and termination in the anterior lobe. *Exp. Brain Res.* 36 (1979) 201-217.



- Engberg, I., J.A. Flatman and K. Kadzielawa. The hyperpolarization of motoneurons by electrophoretically applied amines and other agents. *Acta physiol. scand.* 91 (1974) 2 A - 4 A.
- Falck, B. Observations on the possibilities of the cellular localization of monoamines by a fluorescence methods. *Acta physiol. scand.* 56, suppl. 197 (1962).
- Falck, B., N.-Å. Hillarp, G. Thieme and A. Torp. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem.Cytochem.* 10 (1962) 348-354.
- Feldberg, W. and R.D. Myers. Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol. (London)* 173 (1964) 226-237.
- Fuller, R.W. Selective inhibition of monoamine oxidase. *Adv. Biochem.Psychopharm.* 5 (1972) 339-354.
- Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.* 64, suppl. 247 (1965) 37-85.
- Fuxe, K., T. Hökfelt and U. Ungerstedt. Localization of indoleamines in CNS. *Adv. Pharmacol.* 6 A (1968) 235-251.
- Gallager, D.G. and A. Pert. Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis caudalis and nucleus gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. *Brain Res.* 144 (1978) 257-276.
- Green, A.R. and D.G. Grahame-Smith. Effects of drugs on the processes regulating the functional activity of brain 5-hydroxytryptamine. *Nature* 260 (1976) 487-491.
- Griersmith, B.T. and A.W. Duggan. Prolonged depression of spinal transmission of nociceptive information by 5-HT administered in the substantia gelatinosa: antagonism by inethysergide. *Brain Res.* 187 (1980) 231-236.
- Groenewegen, H.J. and J. Voogd. The parasagittal zonation within the olivo-cerebellar projection. I. Climbing fiber distribution in the vermis of cat cerebellum. *J. Comp. Neurol.* 174 (1977) 417-488.



- Groenewegen, H. J., J. Voogd and S.L. Freedman. The parasagittal zonation within the olivo-cerebellar projection. II. Climbing fiber distribution in the intermediate and hemisphere parts of cat cerebellum. *J. Comp. Neurol.* 183 (1979) 551-602.
- Gwyn, D.G., G.P. Nicholson and B.A. Flumerfelt. The inferior olivary nucleus of the rat: a light and electron microscopic study. *J. Comp. Neurol.* 174 (1977) 489-520.
- Håkanson, R., M.-N. Lombard Des Gouttes and Ch. Owman. Activities of tryptophan hydroxylase, dopa decarboxylase and monoamine oxidase as correlated with the appearance of monoamines in developing rat pineal gland. *Life Sci.* 6 (1967) 2577-2585.
- Headley, P.M. and D. Lodge. Studies on field potentials and on single cells in the inferior olivary complex of the rat. *Brain Res.* 101 (1976) 445-459.
- Heller, A., J.A. Harvey and R.Y. Moore. A demonstration of a fall in brain serotonin following central nervous system lesions in the rat. *Biochem. Pharmacol.* 11 (1962) 859-866.
- Jacobs, B.L. An animal behavior model for studying central serotonergic synapses. *Life Sci.* 19 (1976) 777-786.
- Jacobs, B.L., S.L. Foote and F.E. Bloom. Differential projections of neurons within the dorsal raphe nucleus of the rat: a horseradish peroxidase (HRP) study. *Brain Res.* 147 (1978) 149-153.
- Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. In Ergebnisse der Physiologie, Vol. 64, Neurophysiology and Neurochemistry of Sleep and Wakefulness, Springer-Verlag, Berlin, 1972, pp. 166-308.
- Kawamura, K. and T. Hashikawa. Olivocerebellar projections in the cat studied by means of anterograde axonal transport of labeled amino acids as tracers. *Neuroscience* 4 (1979) 1615-1633.
- Kellar, K.J., P.A. Brown, J. Madrid, M. Bernstein, J. Vernikos-Donellis and W.R. Mehler. Origin of serotonin innervation of forebrain structures. *Exp. Neurol.* 56 (1977) 56-62.
- Komiskey, H.L. and T.A. Rudy. Serotonergic influences on brain stem thermoregulatory mechanisms in the cat. *Brain Res.* 134 (1977) 297-315.
- Krulich, L. Central neurotransmitters and the secretion of prolactin, GH, LH and TSH. *Ann. Rev. Physiol.* 41 (1979) 603-615.





- Kuypers, H.G.J.M. and V.A. Maisky. Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. *Neurosci. Lett.* 1 (1975) 9-14.
- Lamarre, Y. and E. Puil. Induction of rhythmic activity by harmaline. *Canad. J. Physiol. Pharmacol.* 52 (1974) 905-908.
- Larra, F. and B. Droz. Techniques radioautographiques et leur application à l'étude du renouvellement des constituants cellulaires. *Journal de Microscopie* 9 (1970) 845-880.
- Léger, L. and L. Descarries. Serotonergic nerve terminals in the locus coeruleus of adult rat: a radioautographic study. *Brain Res.* 145 (1978) 1-13.
- Lindvall, O. and A. Björklund. The glyoxylic acid fluorescence histochemical method: a detailed account of the methodology for the visualization of central catecholamine neurons. *Histochemistry* 39 (1974) 97-127.
- Llinás, R., R. Baker and C. Sotelo. Electrotonic coupling between neurons in the cat inferior olive. *J. Neurophysiol.* 37 (1974) 560-571.
- Llinás, R. and R.A. Volkind. The olivo-cerebellar system: functional properties as revealed by harmaline-induced tremor. *Exp. Brain Res.* 18 (1973) 69-87.
- Llinás, R., K. Walton, D.E. Hillman and C. Sotelo. Inferior olive.: Its role in motor learning. *Science* 190 (1975) 1230-1231.
- Llinás, R. and Y. Yarom. Electrophysiological properties of mammalian inferior olivary cells in vitro. In J. Courville (Ed.), The Inferior Olivary Nucleus, Raven Press, New York, 1980, in press.
- Loewenstein, W.R. and B. Rose. Calcium in (junctional)intercellular communication and a thought or its behaviour in intracellular communication. *Ann. N.Y. Acad. Sci.* 307 (1978) 285-307.
- Lorens, S.A. and H.C. Guldberg. Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat. *Brain Res.* 78 (1974) 45-56.
- McCall, R.B. and G.K. Aghajanian. Serotonergic facilitation of facial motoneuron excitation. *Brain Res.* 169 (1979) 11-27.



- Messing, R.B. and L. Lytle. Serotonin-containing neurons: their possible role in pain and analgesia. *Pain* 4 (1977) 1-21.
- Montarolo, P.G., F. Raschi and P. Strata. Cerebellar strips and the origin of climbing fibres. *Neurosci. Lett. Suppl.* 3 (1979) S 126.
- Moolenaar, G.-M., J.A. Holloway and C.O. Trouth. Responses of caudal raphe neurons to peripheral somatic stimulation. *Exp. Neurol.* 53 (1976) 304-313.
- Moore, R.Y., A.E. Halaris and B.E. Jones. Serotonin neurons in the midbrain raphe. Ascending projections. *J. Comp. Neurol.* 180 (1978) 417-438.
- Nobin, A., H.G. Baumgarten, A. Björklund, L. Lachenmayer and U. Stenevi. Axonal degeneration and regeneration of the bulbospinal indolamine neurons after 5,6-dihydroxytryptamine treatment. *Brain Res.* 56 (1973) 1-24.
- Nojyo, Y. and Y. Sano. Ultrastructure of the serotonergic nerve terminals in the suprachiasmatic and interpeduncular nuclei of rat brains. *Brain Res.* 149 (1978) 482-488.
- Nygren, L.G., K. Fuxe, G. Jonsson and L. Olson. Functional regeneration of 5-hydroxytryptamine nerve terminals in the rat spinal cord following 5,6-dihydroxytryptamine induced degeneration. *Brain Res.* 78 (1974) 377-394.
- Olson, L. and Å. Seiger. Early prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Z. Anat. Entwickl.-Gesch.* 137 (1972) 301-316.
- Oscarsson, O. and B. Sjölund. The ventral spino-olivocerebellar system in the cat. I. Identification of five paths and their termination in the cerebellar anterior lobe. *Exp. Brain Res.* 28 (1977) 469-486.
- Oscarsson, O. and B. Sjölund. The ventral spino-olivocerebellar system in the cat. II. Termination zones in the cerebellar posterior lobe. *Exp. Brain Res.* 28 (1977) 487-503.
- Palkovitz, M., J.M. Saavedra, D.M. Jacobowitz, J.S. Kizer, L. Záborszky and M.J. Brownstein. Serotonergic innervation of the forebrain: effect of lesions on serotonin and tryptophan hydroxylase levels. *Brain Res.* 130 (1977) 121-134.
- Pearse, A.G.E. *Histochemistry, Theoretical and Applied*. 3rd edit. Vol. 1. Churchill Ltd., London, 1968.





- Phillis, J.W., N. Lake and G. Yarbrough. Calcium mediation of the inhibitory effects of biogenic amines on cerebral cortical neurones. *Brain Res.* 53 (1973) 465-469.
- Raisman, G. Neuronal plasticity in the septal nuclei of the adult rat. *Brain Res.* 14 (1969) 25-48.
- Raisman, G. and P.M. Field. A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res.* 50 (1973) 241-264.
- Reader, T.A., A. Ferron, L. Descarries and H.H. Jasper. Modulatory role for biogenic amines in the cerebral cortex. Microiontophoretic studies. *Brain Res.* 160 (1979) 217-229.
- Rose, B. and W.R. Loewenstein. Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration: A study with aequonin. *J. Membrane Biol.* 28 (1976) 87-119.
- Sakai, K., D. Salvert, M. Touret and M. Jouvét. Afferent connections of the nucleus raphe dorsalis in the cat as visualized by horseradish peroxidase technique. *Brain Res.* 137 (1977) 11-35.
- Sastry, B.S.R. and J.W. Phillis. Antagonism of biogenic amine-induced depression of cerebral cortical neurons by  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase inhibitors. *Canad. J. Physiol. Pharmacol.* 55 (1977a) 170-179.
- Sastry, B.S.R. and J.W. Phillis. Inhibition of cerebral cortical neurones by a 5-hydroxy-tryptaminergic pathway from median raphe nucleus. *Canad. J. Physiol. Pharmacol.* 55 (1977b) 737-743.
- Segal, M. Physiological and pharmacological evidence for a serotonergic projection to the hippocampus. *Brain Res.* 94 (1975) 115-132.
- Segal, M. and S.C. Landis. Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.* 78 (1974a) 1-15.
- Segal, M. and S.C. Landis. Afferents to the septal area of the rat studied with the method of retrograde axonal transport of horseradish peroxidase. *Brain Res.* 82 (1974b) 263-268.



- Seiger, Å. and L. Olson. Late prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Z. Anatl Entwickl.-Gesch.* 140 (1973) 281-318.
- Sjölund, B., L. Wiklund and A. Björklund. Functional role of serotonergic innervation of inferior olivary cells. In J. Courville (Ed.) The Inferior Olivary Nucleus. Raven Press, New York, 1980.
- Sotelo, C., R. Llinás and R. Baker. Structural study of inferior olivary nucleus of the cat: morphological correlates of electrotonic coupling. *J. Neurophysiol.* 37 (1974) 541-559.
- Szabadi, E., C.M. Bradshaw and P. Bevan. Excitatory and depressant neuronal responses to noradrenaline, 5-hydroxytryptamine and mescaline: the role of baseline firing rate. *Brain Res.* 126 (1977) 580-583.
- Taber Pierce, E., G.H. Hoddevik and F. Walberg. The cerebellar projection from the raphe nuclei in the cat as studied with the method of retrograde transport of horseradish peroxidase. *Anat.Embryol.* 152 (1977) 73-87.
- Tohyama, M., K. Sakai, D. Salvert, M. Touret and M. Jouvét. Spinal projections from the lower brainstem in the cat as demonstrated by the horseradish peroxidase technique. I. Origins of the reticulospinal tracts and their funicular trajectories. *Brain Res.* 173 (1979) 383-403.
- Twarog, B.M. and I.H. Page. Serotonin content of some mammalian tissues and urine and a method for its determination. *Am.J.Physiol.* 175 (1953) 157-161.
- Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol.scand. suppl.* 367 (1971) 1-48.
- Van de Kar, L.D. and S.A. Lorens. Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. *Brain Res.* 162 (1979) 45-54.
- Von Hungen, K., S. Roberts and D.F. Hill. Serotonin-sensitive adenylate cyclase activity in immature rat brain. *Brain Res.* 84 (1975) 257-267.
- Voogd, J. The importance of fiber connections in the comparative anatomy of the mammalian cerebellum. In R. Llinás (Ed.) Neurobiology of Cerebellar Evolution and Development, American Medical Association, Chicago, 1969, pp. 493-514.





- Wang, R.Y. and G.K. Aghajanian. Inhibition of neurons in the amygdala by dorsal raphe stimulation: mediation through a direct serotonergic pathway. *Brain Res.* 120 (1977) 85-102.
- Wang, R.Y., D.G. Gallagher and G.H. Aghajanian. Stimulation of pontine reticular formation suppresses firing of serotonergic neurones in dorsal raphe. *Nature* 264 (1976) 365-368.
- Wiklund, L., J.J. Lundberg and K. Møllgård. Species differences in serotonergic innervation and secretory activity of rat, gerbil, mouse and rabbit subcommissural organ. *Acta physiol. scand. suppl.* 452 (1977) 27-30.
- Wiklund, L., B. Sjölund and A. Björklund. Serotonergic innervation of the inferior olive - involvement in tremor mechanisms. *Neurosci. Lett. Suppl.* 1 (1978) S 155.
- Wiklund, L., B. Sjölund and A. Björklund. The inferior olivary nucleus - serotonergic basis for a tremor phenomenon. In B. Schott, M. Devic, M. Tommasi, M. Jouvet, G. Aimard, M. Trillet, C. Quincy and B. Renaud. Les Neuromédiateurs du Tronc Cérébral, Lyon, 1980, in press.
- Windle, W.F. (Editor) Regeneration in the Central Nervous System. Thomas Publ. Co., Springfield, Illinois, 1955.



